高糖誘發人類腹膜間皮細胞引起細胞凋亡路徑中粒線體所扮演的角色

Mitochondria in high glucose induced human peritoneal mesothelial cell apoptosis.

中文摘要

在台灣，因末期腎病（end-stage renal disease, ESRD） 須要接受長期透析或移植治療的患者，約有四萬多人。腎臟病在國人十大死因中佔第七位；而慢性尿毒症的排行中，每年新發生率方面，台灣皆佔世界的第二位。Continuous ambulatory peritoneal dialysis (CAPD) 是末期腎臟衰竭病患治療方式中較方便且不影響病患生活方式，但是長期腹膜透析所引起的腹膜纖維化（peritoneal fibrosis，PF）是腹膜透析病人最常見的合併症之一，嚴重時甚至會影響生命。臨床上發現：目前使用的傳統腹膜透析溶液 (peritoneal dialysis ，PD solution) 具有高糖、高滲透壓、低酸鹼度，等透析液生物相容性不佳的問題。其中高濃度糖為腹膜透析溶液中可成功調整滲透壓達到脫水目的最主要成分。然而，目前台灣常用的糖濃度為 1.5％ (83.8mM)、2.5％ (138mM)及 4.25％ (236mM)三種配製。人類腹膜間皮細胞 （human peritoneal mesothelial cell，HPMC） 長期暴露在這遠超過生理濃度的 glucose 下會改變腹膜的結構與功能，造成人類腹膜間皮細胞逐漸脫落、細胞外間質(extracellular matrix ，ECM) 堆積、腹膜慢性發炎、間皮細胞的凋亡（apoptosis），使腹膜剝離 （detachment）甚至功能衰敗。由先前的文獻指出 2.5%、4.25% glucose 會引起人類腹膜間皮細胞壞死 (necrosis) 或細胞凋亡 (apoptosis)，但是其分子機制引發 HPMC 目前仍未明瞭，尤其粒線體以及活性氧物質所扮演的角色及調控機制也尚未被釐清。本論文研究主旨為高糖引起人類腹膜細胞凋亡路徑中粒線體所扮演的角色，我們推測高糖處理會經由粒線體氧化磷酸化反應（oxidative phosphorylation）產生活性氧自由基 （reactive oxygen species，ROS） 而引發細胞凋亡，我們於本研究發現經過 138mM glucose 及 236mM glucose 處理的人類腹膜細胞會引起細胞凋亡;以西方墨點法可觀察到人類腹膜細胞 cytochrome c release 增加、 PARP cleavage 增加以及 caspase－9 、caspase-3 被活化，並且增加 collagen mRNA 3.66 倍的表現。此外，我們亦進一步觀察，以大鼠環間膜細胞（rat glomerular mesangial cell，RMC）為細胞模式的實驗中將 35mM glucose 添加 1mM l-N-acetylcystein (L-NAC)、粒線體氧化磷酸化抑制劑 1 μM rotenone ；及 1 μM 粒線體去偶合劑 carbonyl cyanide m-chlorophenylhydrazone (CCCP) 可降低高糖刺激對 RMC 造成的傷害及分別減低細胞凋亡 50%、60%、12%。我們的實驗結果可以解釋糖尿病腎臟病的可能原因，以及高糖透析液對 CAPD 患者長期使用對腹膜的不良影響，也可以作為日後研發藥物治療的理論基礎。

英文摘要

In Taiwan, there are at present more than forty thousand patients with end-stage renal disease (ESRD) under regular dialysis therapy. Kidney disease is the seventh of ten leading causes of mortality in our country.The incidence rate of ESRD in Taiwan has gained the second place in the world. Continuous ambulatory peritoneal dialysis (CAPD) is a convenient therqpy as one kind of renal replacement therapy which interferes less of daily activity in ESRD patients. Peritoneal fibrosis (PF) is one of the most common complications in peritoneal dialysis (PD). It has been found that bio-incompatible PD solutions bearing characteristics of high glucose (HG), hyperosmolality, and low pH value may leads to the development of PF. The glucose concentration of PD solutions in Taiwan is 1.5％(83.8mM), 2.5％(138mM), or 4.25％ (236mM). After long-term exposure of HG condition, human peritoneal mesothelial cells (HPMCs) will result in changes of peritoneal structure and function. Histological evaluations had demonstrated that HPMCs apoptosis and accumulation of extracellular matrix (ECM) are main pathogenesis of PF. However, the mechanisms of HG-induced apoptosis of HPMCs and the role of mitochondria as well as reactive oxygen species (ROS) in PF remains undetermined.
This research is aims to evaluate effects of HG in apoptosis of HPMCs. We also investigated the efficacy of mitochondria oxidative phosphorylation and ROS generation in HPMCs under HG condition. It was found that HG induced HPMC apoptosis, cytochrome c release, PARP cleavage, activations of caspase-9, caspase-3, and an up-regulated gene expression of type I collagen with 138mM and 236mM glucose treatment. It was also demonstrated that these detrimental effects of HG in RMC caould be significantly debased apoptosis 50％ by 1mM l-N-acetylcystein (L-NAC), debased apoptosis 60％ by 1μM rotenone or debased apoptosis 12％by 1 μM (CCCP) carbonyl cyanide m-chlorophenylhydrazone supplemented. Induced apoptosis and ECM accumulation were also revealed in rat mesangial cell cuture with 35mM glucose treatment. Our work may have great clinical implications in management of diabetic nephropathy and provide pharmacological information for prevention of PF in long-term PD patients.